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P. P. Somers

The Determination of Sucrose in
Plant Extracts

**THE DETERMINATION OF SUCROSE IN PLANT
EXTRACTS. AN INVESTIGATION OF THE
VARIATION BETWEEN THE VALUES
OBTAINED BY THE POLARISCOPIC
AND CHEMICAL METHODS**

BY

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THESIS

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Acknowledgment

The author wishes here to

express his indebtedness to

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Dr. Duane T. Englis upon whose

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was undertaken. A sincere

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appreciation is felt for his

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of the work and the writing of

ENTITLED. The Determination of Sucrose in Plant Extracts. An Investigation

of the Variation in the Values Obtained by the Polariscope and Chemical
Methods.

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The Determination of Sucrose in Plant Extracts.
An Investigation of the Variation between the Values
Obtained by the Polariscopic and Chemical Methods.

Introduction.

The accurate determination of carbohydrates in plant tissues is a problem of fundamental importance to any study of plant metabolism. In most instances the experimental work which has been carried on, was confined to the determination of certain large groups, such as reducing sugars, and no effort has been made to estimate the quantity of each individual sugar. Recently this problem has aroused considerable interest and several investigators have made definite contributions towards the solution.

It would seem at first glance that of all the sugars which occur in plants in appreciable quantity, sucrose could be the most easily and accurately determined. Since it is a non-reducing sugar and on hydrolysis is converted into dextrose and levulose, both of which are reducing sugars, the chemical method involving a determination of the increase in reducing sugar after hydrolysis can be used for its estimation. Furthermore the fall in rotation accompanying the conversion of the cane sugar to dextrose and levulose affords in the double polarization method, a means of checking the value found by the first mentioned method. Very few investigators in this field of plant chemistry have attempted to check their sucrose values by the two methods, but usually where such an attempt has been made a considerable disparity is observed. Brown and Morris (1)

who are perhaps among the earliest workers to make a detailed study of the individual carbohydrates in plants call attention to this disparity.

The magnitude of the difference between the two values seems to vary with different plant materials and with different portions of the same plant. With nasturtium (*tropaeolum majus*), Brown and Morris report slightly higher values by the polariscopic method. Parkins (2) working with the snow drop (*galanthus nivalis*) states that the polarization value is slightly higher, but usually the difference is negligible. No experimental work is offered to justify his statement. More recently Davis and co-workers (3) in extensive researches on the carbohydrates of the mangold and potato find polarization values which are sometimes as much as eighty per cent greater than the reduction values, while sometimes they are as much as ninety per cent lower. The variation is generally greatest in the stem extracts. Muncie and Englis (4) find with leaf extracts of nasturtium much the same results as Brown and Morris.

Some typical analyses are as follows:

Sucrose	#1	#2	#3	#4
By Polarization Method	99.2	139.2	107.2	97.7
By Reduction Method	94.3	132.3	101.9	92.8

In connection with the determination of Davis and co-workers it may be well to call attention to the fact that they make the common mistake of subtracting copper oxide values before calculating their invert sugar equivalent. As pointed out by Browne (20), low results are obtained. The following recalculation from the data of Davis illustrates this error.

0.3710 G. CuO (after inversion) -- 157.11 mg. invert sugar.

0.2210 G. CuO (before inversion)-- 90.05 mg. invert sugar.

Due to sucrose 67.06 mg. invert sugar.

67.06 mg. invert sugar -- 63.70 mg. sucrose.

Calculation by Davis $\frac{.3710 - .2210}{2.354}(.95) = 60.59$ mg sucrose

63.70 - 60.59 = 3.11 mg. sucrose error.

On this basis, all their reduction values are low and recalculation of their data on the correct basis would tend in cases where the polarization values are high, as they are in most cases, to bring the results by the two methods more nearly together.

The theory advanced by Davis (7) to explain the difference in the values found by the two methods is based on the assumption that certain optically active nitrogenous impurities such as glutamin and asparagin are present which cause a fluctuation in the polarization value. The presence of such impurities and their effect on the determination of sucrose in molasses and cane products has been noted by Pellet (8), Saillard (9), Ogilvie (10) and others. Since the rotation of most of these impurities is altered by the presence of acids, Saillard proposes the determination of sucrose by a double neutral polarization and thus eliminate the disturbance due to the change in rotation of the impurities caused by the addition of the acid to bring about the hydrolysis of the cane sugar. The use of acid and the introduction of neutral salts by neutralization of the acid after hydrolysis can be avoided by carrying out the sucrose inversion by means of the enzyme invertase. Assuming that invertase is specific for the sucrose linkage, it would seem

that if the neutrality of the solution is maintained the rotation of all optically active substances other than sucrose should be of a constant value before and after the inversion and the change in rotation should be due to alteration in the sucrose alone. Consequently the explanation of Davis unless modified is untenable.

Other polysaccharides such as raffinose, gentianose and stachyose contain the sucrose linkage and would be partially hydrolyzed by invertase with an accompanying change in rotation different from that corresponding to the change of sucrose to invert sugar. However, such polysaccharides should also give an increase in reducing power on hydrolysis with acids. No such increase has been noted where proper precautions were taken to prevent enzymatic activity after the picking of the plant material. There is practically no evidence for the occurrence of these higher sugars in the plants which have been investigated so they need not be considered in this connection.

Object of the investigation.

This investigation is an endeavor to find a satisfactory explanation for the difference in the amount of sucrose found by polarization and reduction methods. During the progress of the work several lines of attack have suggested themselves. The experimental work and discussion in connection with each follows.

Experimental Part.

Experiment 1. On the Possibility of the Hydrolysis of Sucrose by Fehling's Solution and Resulting Low Values for Sucrose by the Reduction Method.

(a) Preliminary Discussion: Since in most instances the polarization values were high, the first suggestion by way of explanation of the problem, was that in the determination of reducing sugars before inversion there might be a partial hydrolysis of the sucrose by the Fehling's solution and consequently a resulting low value for sucrose when calculated from the increase in reduction after inversion. Browne (5) has carried out a series of experiments in which he showed that the reduction due to sucrose when accompanying reducing sugars was quite appreciable. Allihn's (11) method, in which the sugar is in contact with the Fehling's solution for two minutes, was used by Browne and he states that the reduction due to the sucrose would be greater in a longer boiling method. Kjeldahl-Woy (12) have worked out a table for the determination of reducing sugars in the presence of sucrose in varying quantities. In this method the heating is continued for twenty minutes but since the process of determination is somewhat involved, it has not found general application. Parkin (2), Davis, Daish and Sawyer (3), and Muncie and Englis (4) have used the method of Brown, Morris and Millar (13) which is one of the longer boiling methods. Parkin (2) claims to have tested various mixtures of hexoses and sucrose and states that the difference due to the sucrose is very small unless present in preponderating proportions. No data is given as

to concentration so it seemed advisable to confirm this statement.

(b) Methods, Materials and Procedure.

Purification of Sucrose: Pure sucrose was prepared by taking a boiling, saturated solution of saccharose and precipitating the sucrose in absolute alcohol which had been refluxed and distilled over lime. The liquid was decanted off and the sucrose precipitate dried at a low temperature in an electric oven. The dextrose used in the experiments was the commercially pure product.

Determination of Reducing Sugars: The Brown-Morris-Millar method was followed in the determination of the reducing sugars. Exactly fifty cubic centimeters of mixed Fehling's solution was pipetted into two hundred fifty cubic centimeters beakers. These beakers were placed in a boiling water bath at two minute intervals. By a series of experiments, it was found that after five minutes in the bath, the beaker and contents would be at a constant temperature. Then fifty cubic centimeters of the sugar was added from a rapid delivering pipette. A rapid delivering pipette was necessary in order to have a homogeneous solution in the minimum time as otherwise layers would be formed and the reduction would not be uniform. Twelve minutes after adding the sugar solution, the beakers were removed and the contents filtered into suction flasks.

It was found that if the filtrate was then taken and the copper determined by the Peter's (14) modification, the process was much more rapid and a greater accuracy was obtained than could be secured by dissolving the cuprous oxide and

determining by Low's method. The filtrates were made up to two hundred cubic centimeters after the addition of five cubic centimeters of concentrated sulphuric acid. A portion was then taken, potassium iodide added and the iodine titrated with sodium thiosulphate, using starch as an indicator. A pure sucrose blank was run with each set of reductions.

Plan of Experiment: Since the sucrose content of most of the plants which have been investigated is seldom more than three times the content of reducing sugars and is usually nearly equal to it, the relative concentrations maintained in this experiment were taken within this range. It seemed advisable, also, to determine whether the quantity of unreduced copper would have any influence in the results.

The experiment was carried out as follows: Two solutions were prepared, the first containing seven grams of dextrose in five hundred cubic centimeters of water and the second containing ten and a half grams of sucrose in two hundred fifty cubic centimeters of water. Portion of each solution were measured carefully from a burette in amounts indicated below and the mixtures made up to one hundred cubic centimeters with water.

	Dextrose	Sucrose	Ratio S/D
1.	50.00	00.00	0
2.	50.00	16.66	1
3.	50.00	50.00	3
4.	28.55	00.00	0
5.	28.55	9.51	1
6.	28.55	28.55	3
7.	14.27	00.00	0
8.	14.27	4.76	1

9. 14.27 14.27 3

Reducing sugars were determined on twenty five cubic centimeter portions.

(c) Results.

Sample	C.C. $\text{Na}_2\text{S}_2\text{O}_3$	CuO equivalent	Dextrose equivalent
1.	5.94	.3708 g.	151.53 mg.
2.	5.94	.3708	151.53
3.	5.92	.3714	151.78
4.	10.86	.2232	87.00
5.	10.70	.2280	89.15
6.	10.87	.2237	87.11
7.	14.97	.0999	38.75
8.	14.57	.1119	43.40
9.	14.97	.0999	38.75
Blank	18.30		

From the results it can be seen that with the amounts of sucrose present there is no apparent increase in the reducing sugars and that the quantity of unreduced copper left in the solution is also without influence. Repetition of the experiment gave similar results.

Experiment 2. The Effect of Neutral Salts on the Rotation of Invert Sugar.

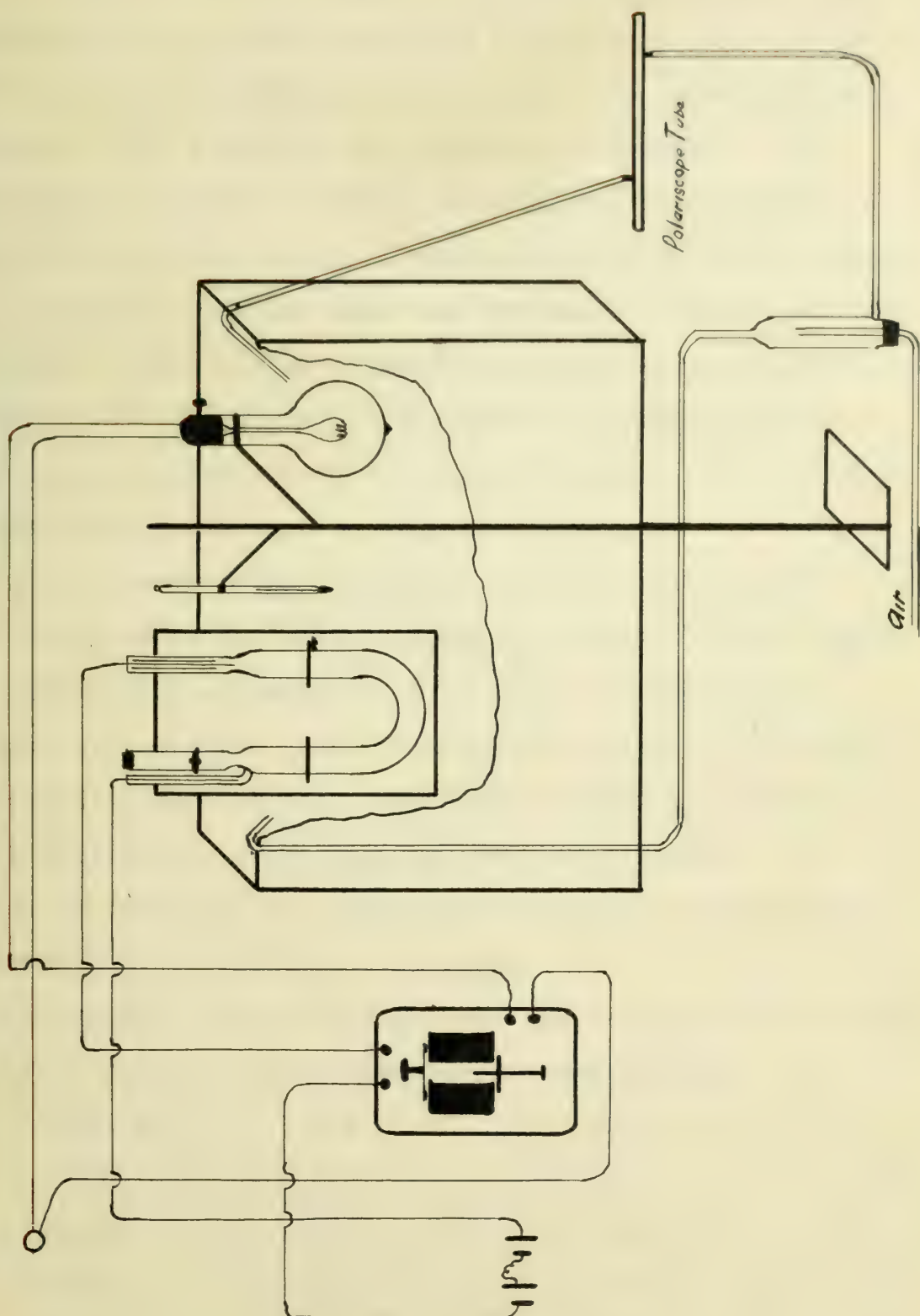
(a) Preliminary Discussion; The second suggestion was that neutral salts such as sodium acetate, which might be left after deleading following the use of basic lead acetate might affect the rotation of sucrose and invert sugar so that the apparent drop in rotation on inversion would be in error and of the nature

to offer a solution of the problem.

Some information is available concerning the effect of neutral salts on sugars. Washburn (15) determined the effect of such salts as sodium chloride, potassium chloride and lithium chloride on the specific rotation of sucrose. In all cases he found that the specific rotation of the sucrose was very slightly decreased by the addition of the salts. Saillard (9) found that the dextro-rotation of the sucrose was decreased by chlorides and hydrochloric acid and that the laevo-rotation of invert sugar was greatly increased. Owing to the very general use of lead acetate as a clearing agent, followed by deleading with sodium carbonate, the principle neutral salt which accumulates is sodium acetate. Saillard does not give any data regarding the effect of neutral acetate salts but merely states that they would act similarly to the chlorides. In order to see if this statement was justifiable, an experiment was planned to secure data concerning the effect of such salts.

(b) Methods, Materials and Procedure

Polarization Apparatus: A Schmidt and Haensch polariscope, a half shaded instrument, calibrated in Ventzke degrees was used. The sugar solutions were polarized in a four decimeter water jacketed tube, maintained at a constant temperature of twenty five degrees Centigrade by the circulation of water from the thermostat. This apparatus is illustrated in the accompanying diagram.



Invertase and Invert Sugar: The cane sugar used in the preparation of the invert sugar was a portion of the sample purified for the prededing experiment and was hydrolyzed with invertase. The invertase was prepared by Sherman's (16) method which consists in taking three pounds of baker's compressed yeast and mixing it thoroughly with fifteen hundred cubic centimeters of tap water and one hundred cubic centimeters of toluene. This mixture, after being allowed to autolyze for five days, was cleaned with lead acetate and filtered, the excess lead being removed by hydrogen sulphide. The solution was then dialyzed through a parchment membrane.

Plan of Experiment: Ten grams of sucrose was placed in a five hundred cubic centimeter volumetric flask. Four hundred cubic centimeters of water and five cubic centimeters of invertase was added. After standing over night, five cubic centimeters of alumina cream was added and the volume made up to five hundred cubic centimeters and then filtered. To Portions of this solution different amounts of sodium acetate were added and the mixtures polarized.

(c) **Results** The following table gives the complete data.

Invert sugar	Sodium acetate	Average reading.
50.00 cc.	0.5 g.	-2.00
50.00	1.0	-2.00
50.00	2.0	-2.00
50.00	3.0	-2.00
50.00	4.0	-2.00

Portions of the solution saturated with sodium oxalate gave an average reading of -2.00.

It seems from the results thus obtained that the amount of sodium acetate or sodium oxalate in the invert sugar solution does not affect the polarization values.

Experiment 3. The Possibility of Impurities Combining with the Sucrose or Invert Sugar and Altering their Rotation Values.

(a) Preliminary Discussion. The third suggestion that impurities either optically or non-optically active, may be responsible for the disparity is indicated in part in an experiment by Davis (17) with mangold extract. To each of two portions of the extract, he added five grams of pure sucrose. After inversion by the invertase and citric acid methods, it was found that only four and ninety eight hundredths grams of the sucrose could be accounted for by the reduction method. On the other hand, the direct polarization value was equal to five and twenty two hundredths grams of sucrose. Davis was working on the methods of extraction and did not try to find the cause of the high values since he was satisfied that no sucrose was lost and correct values could be obtained by the reduction method.

Stanek (18) offers evidence that nitrogenous pigments are formed by the condensation of amino acids with sugar. When solutions of invert sugar or sucrose are heated in an autoclave at one hundred five to one hundred thirty degrees Centigrade with sodium glutamate or aspartate or with asparagin, carbon dioxide is liberated, the solution becomes acidic, and dark pigments are formed which are almost completely precipitated with lead acetate. Asparagin and aspartic acid give rise to

much more of this product than does glutamic acid.

Maillard (19) in an earlier work finds that when glycocoll is warmed with four parts of glucose in three to four parts of water, the liquid slowly assumes a characteristic yellow color, changing to a dark brown and followed by foaming (carbon dioxide). It has been shown that the carbon dioxide came from the carboxyl group of the glycocoll. Then assuming that this loss of carbon dioxide is accompanied by a union of the nitrogen with the aldehyde carbon of the sugar, the glucose molecules forming part of the new compounds, must suffer dehydration resulting in the appearance of double bonds or possibly rings. It was also demonstrated that other amino acids worked the same way on glucose and similarly, various sugars act this way on glycocoll.

Apparently the presence of amino compounds in the plant extracts alter both the direct polarization value of the added sucrose and also that of invert sugar. Since asparagin and glutamin have been found in many plants and are suspected of being the generally occurring nitrogenous impurities, it seemed advisable to determine the effect of the addition of these compounds to sugar solutions.

(b) Methods, Materials and Procedure.

The invert sugar was made up as previously described. The asparagin was from a laboratory stock solution and the apparatus was the polariscopic apparatus previously described. The asparagin and invert sugar solutions were mixed and polarized in accordance with the following table.

(c) Results.

	A	B	C
Asparagin	25 cc.	25 cc.	00 cc.
Invert Sugar	00	25	25
Water	25	00	25
Polarization Reading	-.320	-1.435	-1.060

The mixed asparagin and invert sugar solution give a **greater negative** rotatory value than that obtained by adding the rotation values for the constituents determined separately. This seems to indicate that there is some sort of loose combination between the sugar and amino groups. Under the conditions of low concentration and low temperatures, no formations of pigments or insoluble compounds analogous to those described by Stanek and Maillard was observed so it is hardly possible that the reaction is of the same character. However, it seems plausible that it may be responsible for at least part of the disparity between the two methods for the estimation of sucrose.

An attempt was made to repeat the experiment with a neutral solution of glutamic acid hydrochloride but there was such a development of color that it was impossible to determine the polarization value of the solution.

Summary.

1. There is a disparity between the polariscopic and chemical methods for the determination of sucrose in plant extracts.

2. In the concentration in which sucrose is present in plant extracts, there is no hydrolysis due to the Fehling's solution which would result in low values for sucrose by the reduction method.

3. Neutral salts such as sodium acetate and sodium oxalate, do not have any effect on the rotation of the invert sugar.

4. As an impurity, Asparagin causes an increased value for the sucrose by the polarization method and serves as one explanation for the disparity between the two methods.

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